

Claims

1. Process for the production of ergosterol and its intermediate products, characterized in that

- a) first a plasmid is designed, into which several suitable genes of the ergosterol metabolic process are inserted in altered form,
- or
- b) first plasmids are designed, into which in each case one of the genes of the ergosterol metabolic process is inserted in altered form,
- c) microorganisms are transformed with the thus produced plasmids, whereby the microorganisms are transformed with a plasmid under a) or they are transformed simultaneously or in succession with several plasmids under b),
- d) fermentation into ergosterol is performed with the thus produced microorganisms,
- e) after fermentation has ended, the ergosterol and its intermediate products are extracted from the cells and analyzed, and finally,
- f) the thus obtained ergosterol and its intermediate products are purified using column chromatography and isolated.

2. Process according to claim 1, wherein

a-i) first a plasmid is designed, into which the following genes are inserted:

- i) the gene of HMG-Co-A-reductase (t-HMG),
- ii) the gene of squalene synthetase (ERG9),
- iii) the gene of Acyl-CoA: sterol-acyl transferase (SAT1),

and

- iv) the gene of squalene epoxidase (ERG1),

or

a-ii) first a plasmid is designed, into which the following genes are inserted:

- i) the gene of HMG-Co-A-reductase (t-HMG),

and

- ii) the gene of squalene synthetase (ERG9),

or

a-iii) first a plasmid is designed, into which the following genes are inserted:

- i) the gene of HMG-Co-A-reductase (t-HMG),

and

- iii) the gene of acyl-CoA: sterol-acyl transferase (SAT1),

or

a-iv) first a plasmid is designed, into which the following genes are inserted:

- i) the gene of the HMG-Co-A-reductase (t-HMG),

and

iv) the gene of squalene epoxidase (ERG1),

or

a-v) first a plasmid is designed, into which the following genes are inserted:

ii) the gene of squalene synthetase (ERG9),

and

iii) the gene of acyl-CoA: sterol-acyl transferase
(SAT1)

or

a-vi) first a plasmid is designed, into which the following genes are inserted:

ii) the gene of squalene synthetase (ERG9),

and

iv) the gene of squalene epoxidase (ERG1),

or

a-vii) first a plasmid is designed, into which the following genes are inserted:

iii) the gene of acyl-CoA: sterol-acyl transferase
(SAT1),

and

iv) the gene of squalene epoxidase (ERG1),

or

b) first plasmids are designed, into which in each case one of the genes that is mentioned under a-i) is inserted,

and

- c) microorganisms are transformed with the thus produced plasmids, whereby the microorganisms are transformed with a plasmid under a-i) to a-vii), or they are transformed simultaneously or in succession with several plasmids under b),
- d) fermentation into ergosterol is performed with the thus produced microorganisms,
- e) after fermentation has ended, the ergosterol and its intermediate products are extracted from the cells and analyzed, and finally
- f) the thus obtained ergosterol and its intermediate products are purified using column chromatography and isolated.

3. Process according to claim 2, wherein in addition the gene of squalene epoxidase (ERG1) is inserted into the plasmid under a-ii), a-iii) and a-v), and in addition the gene of the acyl-CoA: sterol-acyl transferase is inserted into plasmid a-ii).

4. Process for the production of ergosterol and its intermediate products, wherein the genes that are mentioned in claim 1 under a), those in claim 2 under a-i) to a-vii) and those in claim 3 under a-ii), a-iii) and a-v) in each case with the plasmids are first introduced independently of one another into microorganisms of the same species, and fermentation into ergosterol is performed with them together and the ergosterol that is thus obtained is extracted from the cells, analyzed and purified using column chromatography and isolated.

5. Process according to claims 1 to 4, wherein the intermediate products are squalene, farnesol, geraniol, lanosterol, zymosterol, 4,4-dimethylzymosterol, 4-methylzymosterol, ergost-7-enol and ergosta-5,7-dienol.

6. Process according to claims 1 to 4, wherein the intermediate products are sterols with 5,7-diene structure.

7. Process according to claims 1 to 4, wherein the plasmids are plasmids YEpH2, YDpUHK3 and pADL-SAT1.

8. Process according to claims 1 to 4, wherein the microorganisms are yeasts.

9. Process according to claim 8, wherein it is the species *S. cerevisiae*.

10. Process according to claim 9, wherein it is the strain *S. cerevisiae* AH22.

11. Yeast strain *S. cerevisiae* AH22 that contains one or more of the genes that are mentioned under a-i) in the process.

12. Plasmid YEpH2 that consists of the average ADH-promoter, t-HMG (altered variant of HMG-1) and the TRP-terminator (Fig. 1).

13. Plasmid YDpUHK3 that consists of the average ADH-promoter, t-HMG (altered variant of the HMG-1) and the TRP-terminator, the gene for the kanamycin resistance and the *ura3* gene (Fig. 2).

14. Plasmid pADL-SAT1 that consists of the SAT1 gene and the LEU2 gene of YEp13.

15. Use of the plasmids according to claims 12 to 14 for the production of ergosterol.

16. Use of the plasmids according to claims 12 to 14 for the production of ergosterol intermediate products squalene, farnesol, geraniol, lanosterol, zymosterol, 4,4-dimethylzymosterol, 4-methylzymosterol, ergost-7-enol and ergosta-5,7-dienol.

17. Use of the plasmids according to claims 12 to 14 for the production of sterols with 5,7-diene structure.

18. Expression cassette that comprises the average ADH-promoter, the t-HMG gene, the TRP-terminator and the SAT1 gene with the average ADH-promoter and the TRP-terminator.

19. Expression cassette that comprises the average ADH-promoter, the t-HMG gene, the TRP-terminator, the SAT1 gene with the average ADH-promoter and the TRP-terminator, and the ERG9-gene with the average ADH-promoter and the TRP-terminator.

20. Combination of expression cassettes, whereby the combination consists of

- a) a first expression cassette, on which the ADH-promoter, the t-HMG-gene, and the TRP-terminator are located,
- b) a second expression cassette, on which the ADH-promoter, the SAT1-gene and the TRP-terminator are located,

and

- c) a third expression cassette, on which the ADP-promoter, and the ERG9-gene with the TRP-terminator are located.

21. Use of the expression cassettes according to claims 18 to 20, for the transformation of microorganisms, which are used in the fermentation into ergosterol.

22. Use according to claim 21, wherein the microorganism is yeast.

23. Microorganisms that contain expression cassettes according to claims 18 to 20.

24. Microorganism according to claim 23, wherein it is yeast.

25. Use of the microorganism according to claims 23 and 24 in the fermentation into ergosterol.

26. Use of the microorganism according to claims 23 and 24, in the fermentation into ergosterol intermediate products.